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Genomic organization of the mouse T cell receptor Vα family

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Based on the analysis of Vα gene segment deletions in a panel of T lymphomas, we have constructed a map of the mouse T cell receptor α/δ region and assigned the relative position of 72 distinct V gene segments. Three major observations have emerged from such studies. First, members of a given Vα subfamily are not organized in discrete units along the chromosome but largely interspersed with members of other Vα subfamilies. Second, analysis of the deletion map suggests the existence of repetitive patterns (Vα clusters) in the chromosomal distribution of the Vα gene segments. Third, the present-day organization of the Vα/δ region may be readily explained by a series of sequential duplications involving three ancestral Vα clusters. Direct evidence for the existence of these unique structural features has been gained by cloning ~370 kb of DNA and positioning 26 distinct Vα gene segments belonging to six different subfamilies. Finally, the relationships existing between the Vα/δ gene segment organization and usage are discussed in terms of position-dependent models.

Key words: gene rearrangement/mouse T cell receptor/repertoire/Vα gene segment organization

Introduction

Mouse T cells can be divided into two subsets based on the structure of their specific receptors for antigen (T cell receptor, TCR). In the adult, most peripheral T cells express a TCR heterodimer consisting of α and β chains, whereas a small percentage (1 - 10%) of peripheral T cells and a majority of intraepithelial T lymphocytes express an alternative TCR form made of γ and δ chains (reviewed in Davis and Bjorkman, 1988). Each of these four TCR chains includes one variable (V) and one constant (C) region. As previously observed for the immunoglobulin heavy and light chain genes, functional TCR V genes are formed by the somatic rearrangement and juxtaposition of variable (V), diversity (D, in the case of TCR β and TCR δ) and joining (J) gene segments. The organization of the α and δ gene elements displays unique structural features among antigen receptor genes. First, α and δ genes belong to the same genetic complex encoded on chromosome 14 (Chien *et al.*, 1987a). Second, the Dδ and Jδ gene segments and the Cδ gene are located between the Vα and Jα gene segments (Chien *et al.*, 1987b). Most of the Vα-Jα rearrangements have been shown to result in the excision of the Dδ-Jδ-Cδ cluster (Chien *et al.*, 1987a: Fujimoto and Yamagishi, 1987; Lindsten *et al.*, 1987; Okazaki and Sakano, 1988). As a consequence, the majority of the Vα gene segments have therefore been postulated to map at an undetermined distance 5′ to the Dδ1 gene segment and to rearrange via deletional mechanisms.

Based on the analysis of V segment usage in cloned T cells, the mouse Vα family has been estimated to comprise approximately 75 - 100 members (Arden *et al.*, 1985; Becker *et al.*, 1985). These V gene segments can be grouped into distinct subfamilies based upon sequence similarity. V gene segments that show >75% similarity at the nucleotide level are considered members of the same subfamily (Brodeur and Riblet, 1984). Operationally, members of a given subfamily can be identified on Southern blots by hybridization to a prototypic V gene segment probe. Southern blot analyses have indicated that the Vα subfamilies identified contain from one to ten members (Arden *et al.*, 1985). Furthermore, analyses of restriction fragment length polymorphism of TCR Vα gene segments among different inbred mouse strains have suggested that the members of a given subfamily are not organized in discrete clusters but instead are probably interspersed with members of other subfamilies (Singer *et al.*, 1988; Jouvin-Marche *et al.*, 1989; Klotz *et al.*, 1989). The unique organization of the α and δ gene elements raises the possibility that a shared pool of V gene segments is used to produce both α and δ chain genes. There is indeed some overlap in V segment usage: seven of the 11 identified Vδ gene segment subfamilies (Elliot *et al.*, 1988; Raulet, 1989; Takeshita *et al.*, 1989) overlap with or are identical to known Vα subfamilies. However, four of the Vδ subfamilies have not been observed in surveys of large numbers of α genes and are possibly exclusively used to produce δ chain genes (see Discussion).

To study the organization of the mouse Vα/δ gene segment families, we have used two complementary approaches. First we have exploited the fact that most Vα-Jα rearrangements occur via deletion (see above). On a chromosome that has experienced a Vα-Jα deletional rearrangement, the Vα gene segments located between the rearranging Vα and Jα gene segments are lost. In a single T cell, if both copies of chromosome 14 have undergone a Vα-Jα deletional rearrangement, the identity of the Vα gene segments proximal and distal to the 3′ most rearranged Vα gene segment can be determined by Southern blot analysis of the T cell genomic DNA with various Vα-specific probes. Accordingly, we have analysed a panel of seven independently derived T lymphomas which have each rearranged different Vα gene segments on both homologous chromosomes, and constructed a deletion map indicating the relative order of Vα gene segments along the chromosome.

During their *in vitro* propagation, three of the lymphomas have undergone secondary rearrangements that have replaced the pre-existing Vα−Jα rearrangement by joining an upstream Vα to a downstream Jα gene segment (Marolleau *et al.*, 1988). The occurrence of these secondary rearrangements has allowed us to refine further the relative order of some contiguous Vα gene segments. Secondly, to support the salient structural features outlined during deletion mapping, we have directly analysed by phage and cosmid cloning ~370 kb of DNA and positioned 26 distinct Vα gene segments belonging to six different subfamilies.

Results

Vα gene segment rearrangements and deletions in a panel of T lymphomas

DNAs from seven independently derived BALB/c T lymphomas were first analysed by Southern blot using a probe corresponding to the Cδ gene. None of them had retained the Cδ gene (data not shown). As will be substantiated below, these data indicate that in each line both chromosomes 14 have undergone Vα−Jα joining events resulting in the deletion of the Dδ−Jδ−Cδ gene elements. To delineate the nature of the two Vα−Jα rearrangements affecting each of the seven T lymphoma lines as well as to map the extent of the corresponding Vα/δ deletions, DNAs from these lines and BALB/c liver were separately digested with *Eco*RI, *Bam*HI and *Hin*dIII, and analysed with a Vδ1 probe and 18 different Vα probes (see Table I and Materials and methods).

As shown in Figure 1 and summarized in Table II, each T lymphoma has rearranged two distinct Vα gene segments and displays unique patterns of Vα restriction enzyme fragments (REF). Two additional points should be made. First, despite the use of probes specific for 18 Vα subfamilies and the analysis of three sets of endonuclease digests, we have been unable to identify the nature of the second Vα−Jα rearrangement affecting the M31T cell line. However, we have observed the presence in M31T of two distinct rearranged Jα gene segments (data not shown). This observation, together with the fact that the Dδ−Jδ−Cδ region is deleted, suggests that the second Vα−Jα rearrangement present in the M31T T cell line involves a Vα gene segment which has not yet been described. Second, since all the T lymphomas used are derived from the BALB/c strain, which is homozygous for the TCR α/δ locus, only the deletions shared by both chromosomes 14 are detectable on Southern blots. Therefore, the identity of the V gene segments located between the two rearrangement breakpoints and retained in germ-line configuration on the chromosome bearing the 5′ most rearranged Vα gene segment cannot be determined (see Brodeur *et al.*, 1988). However, the fact that three of the T lymphomas analysed (M8T, M14T and M31T) have undergone secondary Vα−Jα rearrangements has allowed us to delineate further the relative Vα order. In these instances, it has been possible to determine the identity of the Vα gene segments localized between the primary and the secondary rearrangement breakpoints (see legend to Figure 1).

Deletion mapping of the Vα gene segment family

The fact that all of the T lymphomas analysed have deleted the single-membered Vα6 and Vδ1 subfamilies (data not shown) suggests that they are the most 3′ of the identified subfamilies. In contrast, at least one copy of the single-membered VαA10 and Vα5T subfamilies has been retained in germ-line configuration in each of the seven T lymphoma lines (data not shown). Therefore, these two Vα subfamilies

Table I. Characteristics of the Vα and Vδ probes used in this study

V subfamily	Origin	Restriction fragment	Size (bp)	Content	References
Vα1[a]	Rearranged genomic clone	*Fok*I−*Mlu*I	340	V	Hue *et al.*, 1990
Vα2[a]	Rearranged genomic clone	*Bam*HI	800	L,V	Hue *et al.*, 1990
Vα3[a]	cDNA	*Pst*I−*Xba*I	1000	V,J,C	Saïto *et al.*, 1984
Vα4[a]	cDNA	*Pst*I−*Eco*RI	346	L,V	Yague *et al.*, 1988
Vα5[a]	cDNA	*Eco*RI−*Pvu*II	270	L,V	E.Palmer, unpublished
Vα6[a]	cDNA	*Eco*RI−*Acc*I	200	L,V	E.Palmer, unpublished
Vα7[a]	cDNA	*Eco*RI−*Not*I	270	L,V	Yague *et al.*, 1988
Vα8[a]	cDNA	*Eco*RI−*Pst*I	280	L,V	Yague *et al.*, 1988
Vα9[a]	cDNA	*Rsa*I	300	L,V	E.Palmer, unpublished
Vα10[a]	Rearranged genomic clone	*Sst*I−*Pst*I	200	V	Hue *et al.*, 1990
Vα11[a]	Rearranged genomic clone	*Pvu*II	580	L,V	Malissen *et al.*, 1988
VαA10	Rearranged genomic clone	*Pst*I−*Sst*I	230	V	Malissen *et al.*, 1988
Vα13[a]	cDNA	*Eco*RI−*Sau*3A	493	L,V	M.Kronenberg and L.Hood, unpublished
Vα14	Rearranged genomic clone	*Eco*RI−*Hin*cII	477	L,V	Koseki *et al.*, 1989
VαBW.B.	Rearranged genomic clone	*Eco*RI−*Bam*HI	400	L,V	Letourneur and Malissen. 1989
VαBM.A[b]	Rearranged genomic clone	*Xba*I−*Eco*RI	429	L,V,J	D.Couez and B.Malissen, unpublished
VαBM.B[c]	Rearranged genomic clone	*Rsa*I−*Mlu*I	234	L,V,J	D.Couez and B.Malissen, unpublished
Vα5T	cDNA	*Eco*RI−*Eco*RV	310	L,V	P.N.Marche, unpublished
Vδ1[d]	cDNA	*Eco*RI−*Eco*RV	430	L,V	Korman *et al.*, 1988

For each V segment, we have indicated the origin, nature and content of the restriction fragment used as a probe. The following abbreviations are used: L, leader; V, variable gene segment; J, joining gene segment; C, constant gene.
[a]Nomenclature according to Arden *et al.*, 1985.
[b]The coding region of VαBM.A gene segment is 98% identical at the nucleotide level to the VαP14A.1 gene segment (Pircher *et al.*, 1987).
[c]The coding region of the VαBM.B gene segment is 98% identical at the nucleotide level to the Vα 5.3.18 gene segment (Sherman *et al.*, 1987).
[d]Nomenclature according to Elliott *et al.*, 1988.

probably map at the distal end of the Vα locus. Two hybridizing fragments were detected when *Eco*RI- or *Bam*HI-digested BALB/c liver DNAs were probed with the VαBW.B gene segment. Since only one of the two VαBW.B hybridizing fragments was retained in all of the T lymphomas tested (Table II), we conclude that the members of the VαBW.B subfamily are non-contiguous and probably localized towards each extremity of the Vα/δ locus. The same line of reasoning was used to analyse the remaining Vα subfamilies (see Figure 1). This allowed the determination of the deletion profiles shown in Table II and yielded a relative chromosomal order of Vα gene segments (Figure 2) that is consistent with all observed patterns of Vα gene segment deletion and retention.

The Vα family is composed of repeated Vα clusters

Two striking points emerge from the analysis of the deletion map shown in Figure 2. First, members of a given Vα subfamily are not organized in discrete sets but largely interspersed with members of other subfamilies. Second, most of the Vα gene segments may be readily organized in three distinct clusters (see Discussion). For instance, as shown in Figure 2, the largest one, denoted as cluster I, is composed of at least eight different Vα gene segments (Vα1, Vα2, Vα3, Vα4, Vα7, Vα8, Vα10 and Vα11) and repeated at least four times throughout the BALB/c Vα/δ locus (clusters Ia, Ib, Ic, Id and possibly Ie in Figure 2). The two other clusters (denoted as II and III in Figure 2) are smaller and include only two to three different Vα gene segments (Vα10, Vα2 and VαBM.B in cluster II; VαBM.A and Vα3 in cluster III). Furthermore, deletion analysis permits delineation of the relative order of some of the Vα gene segments within the four cluster I repeats. For instance, analysis of clusters Ia and Ic, suggests that the Vα gene segments 4-1-8 and 7-11-3 are located on both ends of a repeat and bracket the Vα gene segments 10 and 2.

Genomic organization of the Vα2 subfamily and of its neighbouring Vα gene segments

To obtain direct physical evidence for the existence of the clusters outlined throughout deletion analysis and to define the transcriptional polarity of the various Vα gene segments, genomic clones corresponding to different sections of the Vα locus were subsequently isolated and analysed.

Genomic clones hybridizing to a Vα2-specific probe were isolated from a λ phage library constructed from B10.A liver DNA. The fact that B10.A is a $V\alpha^b$ haplotype strain (Jouvin-Marche *et al.*, 1989) should allow us to extend to an additional Vα haplotype, the results of the deletion analysis obtained on T cell lines derived from a $V\alpha^a$ haplotype. Based on restriction mapping, the 80 isolated phages were organized in nine distinct classes. As shown in Figure 3, representative phages were selected for each class and further mapped with five different restriction enzymes. To determine if the nine distinct Vα2 gene segments contained in this set of phages represent the complete Vα2 subfamily, the sizes of the *Eco*RI fragments hybridizing with a Vα2 probe and present in the nine phage classes were compared with the Vα2 hybridizing *Eco*RI fragments seen in a genomic Southern blot of B10.A liver DNA. As shown in Figure 4, an *Eco*RI digest of an equimolar mixture of representative phages belonging to each of the nine distinct classes fully reconstitutes the genomic Southern blot banding pattern. In the process of analysing the α-chain gene rearrangements in a B10.BR-derived cytolytic T cell clone, we have defined a DNA probe (probe W) specific for sequences located ~2 kb to the 5′ side of three distinct Vα2 gene segments (Hue *et al.*, 1990). As shown in Figure 3, hybridization of probe W to the nine phage classes split them into W-positive (class 5, 6 and 9) and W-negative (class 1, 2, 3, 4, 7 and 8) subsets. In agreement with our previous studies, only three Vα2 gene segments are associated with probe W. Therefore, altogether these data indicate that the nine isolated phage classes probably represent the complete Vα2 subfamily encoded by the $V\alpha^b$ haplotype.

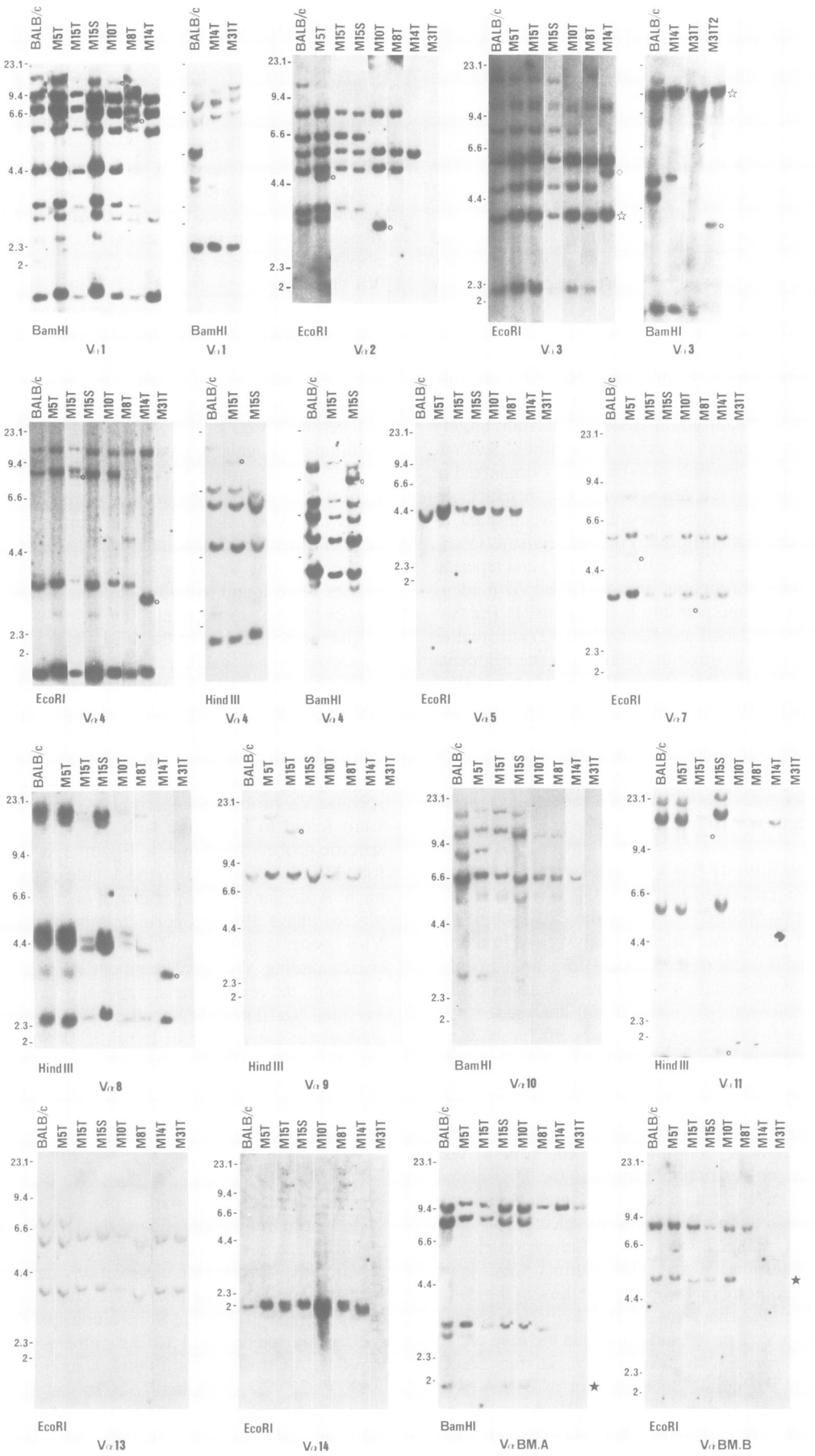
To determine if other Vα gene segments are located in close contiguity to the different member of the Vα2 gene subfamily, phages corresponding to the nine Vα2 classes were hybridized with a panel of probes specific for 18 different Vα subfamilies (see Table I). All the phages isolated with Vα2 probe were found to contain at least one additional Vα gene segment belonging to either the Vα1, Vα7, Vα8, Vα10 or VαBM.B subfamilies. As shown in Figure 3, a Vα10 gene segment was present in eight out of the nine Vα2 classes analysed. Furthermore, a Vα7 gene segment was found associated with five out of the nine Vα2 classes. Among the W-positive subset, the fifth and ninth classes were found to hybridize to a VαBM.B and a Vα1 specific probe, respectively.

The molecular map of the third phage class was extended by chromosomal walking. The probe used for that purpose corresponded to a Vα8 gene segment isolated from the 5′ end of the phage clone 20.2. This procedure yielded the partially overlapping phage clone 25E and allowed us to extend the map ~20 kb to the 5′ side of the Vα2 gene segment. When separately analysed with the 18 subfamily specific Vα probes, Southern blots of the 25E phage clone were found to hybridize with the Vα1, Vα8 and Vα10 specific probes. As shown in Figure 3, a Vα1 segment was found localized ~19 kb to the 5′ side of the Vα2 gene segment. A similar analysis performed on the first class of phage yielded phage 19E and permitted the physical linkage of a Vα8 gene segment ~11 kb to the 5′ side of the corresponding Vα2 gene segment (Figure 3).

Finally, the relative transcriptional orientation of the Vα2, Vα7 and Vα10 gene segments present within the nine Vα2 classes was determined by hybridization with DNA probes specific for 5′ or 3′ flanking regions. As shown in Figure 3, the Vα2, Vα7 and Vα10 gene segments identified in each class were found in the same transcriptional orientation.

Discussion

We have constructed a partial physical map of the mouse TCR Vα/δ family and assigned the relative positions of 72 distinct V gene segments belonging to 18 Vα and one Vδ subfamilies. Two major observations have emerged from such studies. First, members of a given Vα subfamily are not organized in discrete units along the chromosome but are largely interspersed with members of other Vα subfamilies. As illustrated in Figure 3, unique members of the Vα1, Vα2, Vα7, Vα8 and Vα10 subfamilies may be found interspersed within a 30 kb DNA segment. Furthermore, the results of the deletion mapping shown in Figure 2 and the isolation of phage clones containing pairs of Vα gene


BALB/c M5T M15T M15S M10T M8T M14T
BamHI
Vα1
BALB/c M14T M31T
BamHI
Vα1
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα2
BALB/c M5T M15T M15S M10T M8T M14T
EcoRI
Vα3
BALB/c M14T M31T M31T2
BamHI
Vα3
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα4
BALB/c M15T M15S
Hind III
Vα4
BALB/c M15T M15S
BamHI
Vα4
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα5
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα7
BALB/c M5T M15T M15S M10T M8T M14T M31T
Hind III
Vα8
BALB/c M5T M15T M15S M10T M8T M14T M31T
Hind III
Vα9
BALB/c M5T M15T M15S M10T M8T M14T M31T
BamHI
Vα10
BALB/c M5T M15T M15S M10T M8T M14T M31T
Hind III
Vα11
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα13
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
Vα14
BALB/c M5T M15T M15S M10T M8T M14T M31T
BamHI
VαBM.A
BALB/c M5T M15T M15S M10T M8T M14T M31T
EcoRI
VαBM.B
23.1 9.4 6.6 4.4 2.3 2

segments belonging to the Vα1−Vα4, Vα3−Vα8, Vα4−Vα9 and Vα3−Vα11 subfamilies (data not shown) clearly indicate that members of the Vα3, Vα4, Vα9 and Vα11 subfamilies are also interspersed on the chromosome. Secondly, analysis of the deletion map (Figure 2) suggests the existence of repetitive patterns in the chromosomal distribution of the Vα gene segments. These discrete sets of Vα gene segment have been denoted as Vα clusters. Three distinct clusters are readily observable in the $V\alpha^a$ haplotype (see Figure 2 legend). Cluster I is repeated at least four times along the chromosome and contains one member of each of the Vα1, Vα2, Vα3, Vα4, Vα7, Vα8, Vα10 and Vα11 subfamilies. Cluster II is repeated twice and contains one member of each of the Vα2, Vα10 and VαBM.B subfamilies. The 5′ flanking sequences of the Vα2 gene segments belonging to cluster II specifically hybridized to a probe denoted W and are therefore readily distinguishable from the ones belonging to the cluster I. Finally, cluster III is repeated at least three times and contains one member of each of the Vα3 and VαBM.A subfamilies. Direct evidence for the existence of the clusters I and II has been gained by determining the identity of the Vα gene segments flanking each member of the Vα2 subfamily (Figure 3). In that process, we have found at least four distinct repeats of the Vα10-Vα2-Vα7 motif (class 1, 2, 3 and 4 in Figure 3). Moreover, a limited extension of two molecular maps yielded

Fig. 1. Southern blot analysis of T cell receptor Vα gene segments in seven T lymphomas. DNAs from the seven T lymphomas and BALB/c liver (germline) were digested with BamHI, *Eco*RI or *Hin*dIII and, after gel electrophoresis and transfer, separately hybridized to probes specific for 14 distinct Vα subfamilies. *Hin*dIII-digested λ phage DNA was used as a size marker. In the C.B20 derived M14T DNA, the Vα3 hybridizing *Eco*RI fragment marked with an open lozenge migrates at the same position as one C.B20 germline Vα3 gene segment (data not shown) and is therefore assumed to be in germline configuration. The *Bam*HI and *Eco*RI restriction fragments hybridizing to the Cα gene sequences present in the Vα3 probe (Table I) are indicated by an open star. The restriction fragments hybridizing to the J gene segment sequences present in the VαBM.A and VαBM.B probes are marked with a closed star. In the M8T DNA, the additional 5 kb band detected with the Vα4 probe is due to cross-reaction of contaminating plasmid vector sequences with the pBR322-derived sequences present in the provirus harboured by the M8T lymphoma (Primi *et al.*, 1988). **M31T**. This line has rearranged one VαA10 gene segment and retained in germline configuration the single-membered VαA10 and Vα5T subfamilies, a few members of the Vα1 Vα3 and Vα13 subfamilies as well as one member of the VαBM.A and VαBW.B subfamilies. The fact that the M31T cell lines has not retained the complete set of Vα1, Vα3, Vα13 and VαBM.A gene segments suggests that the members of these subfamilies map to different regions of the Vα/δ locus. Among 20 subclones of the M31T cell line tested for secondary rearrangements, one of them (M31T-2) has undergone a single secondary rearrangement detectable with the Vα3-specific probe. Relative to the parental M31T cell line, M31T-2 has conserved the rearranged and the germline VαA10 gene segments and lost only one germline Vα3 hybridizing fragment corresponding to the one involved in the secondary Vα-Jα rearrangement. Taken together, these results indicate that one member of the Vα3 subfamily maps downstream of a group of Vα gene segments containing six members of the Vα1 subfamily, two members of the Vα13 subfamily and one member of each of the Vα5T, VαA10, Vα3, VαBM.A and VαBW.B subfamilies. **M14T**. This line has rearranged a Vα4 and a Vα8 gene segment. Relative to the M31T cell line, M14T has retained from one to two members of seven additional Vα subfamilies as well as new germline fragments corresponding to the Vα1 and Vα3 subfamilies. The comparison of the patterns of hybridization between the M14T cell line and those present in four M14T-derived subclones showed that the latter have lost the initial rearranged Vα8 gene, all the members of the Vα2, Vα7, Vα10 and Vα11 subfamilies and one member of the Vα3 subfamily (Table II). Furthermore, the M14T-2 subclone has deleted one member of the Vα1, Vα4, Vα8 and Vα14 subfamilies. This subclone has conserved the rearranged Vα4 gene segment present in the parental M14T cell line and rearranged one VαBM.A gene segment. Finally, the M14T-2 subclone has retained one member of the Vα4 subfamily relative to the M31T cell line. Taken together these data indicate that the members of the nine distinct subfamilies which were deleted during the M14T-2 secondary rearangement are clustered and localized between the rearranged Vα4 and Vα8 gene segments of the M14T cell line. In three additional M14T subclones (M14T-1, M14T-7 and M14T-8), secondary Vα−Jα rearrangements have occurred on both chromosomes 14. The M14T-1 subclone exhibits two new Vα rearrangements detectable with the Vα8- and Vα3-specific probes. The M14T-7 subclone contains a novel rearranged Vα8 gene segment and displays the same VαBM.A rearrangement as the one observed in the M14T-2 subclone. The M14T-1 and M14T-7 subclones show hybridization patterns identical to those found in the M14T cell line using the Vα1, Vα4 and Vα14 probes. These observations imply that one member of the Vα8 family maps upstream of a group of Vα gene segments containing one member of the Vα2, Vα3, Vα7, Vα10 and Vα11 subfamilies. When compared with the M14T-subclone, the M14T-8 subclone has rearranged one member of the Vα3 and V4α1 subfamilies, and lost two germline Vα4 gene segments and one member of the Vα1, Vα3 and Vα14 subfamilies. This last result strongly suggests that two Vα4 gene segments and one member of the Vα1 and Vα14 subfamilies map between the rearranged Vα3 and Vα8 gene segments of the M14T-1 subclone. **M8T**. This line has rearranged a Vα1 and a Vα3 gene segment and retained one additional member of each of the Vα5, Vα9 and VαBM.B subfamilies relative to the M14T and M31T cell lines. Furthermore, the hybridization patterns observed with the probes Vα1, Vα2, Vα3, Vα4, Vα7, Vα8, Vα10, Vα11 and VαBM.A appear more complex in the M8T cell line than those observed in the M14T cell line. One of the M8T subclones (M8T-7) has conserved the original Vα3 rearrangement and replaced the initial rearranged Vα1 gene segment via a secondary rearrangement detectable with the VαBM.B specific probe. In that process, at least one member of each of the Vα2, Vα7, Vα10 and VαBM.B subfamilies has been deleted. These data indicate that one member of each of the Vα2, Vα7 and Vα10 subfamilies maps in between the rearranged Vα1 gene segment of the M8T cell line and the rearranged VαBM.B gene segment of the M8T-7 subclone, thus defining a group of gene segments containing members of the Vα1, Vα2, Vα7, Vα10 and VαBM.B subfamilies. Comparison of the REF obtained with the M14T cell line and M8T-7 subclone indicates that one member of the Vα1, Vα2, Vα4, Vα5, Vα7, Vα8, Vα9, Vα10, Vα11 and VαBM.A subfamilies and two members of the Vα3 subfamily are linked in a discrete subregion localized between the M14T rearranged Vα8 gene segment and the M8T-7 rearranged VαBM.B gene segment. **M10T**. Relative to the REF pattern observed in the M8T lymphoma, the M10T lymphoma displays one additional member of each of the Vα1, Vα3, Vα4, Vα8, Vα10 and VαBM.A subfamilies. One M10T Vα rearrangement is detectable with the Vα1 specific probe and the other with both the Vα2 and Vα7 specific probes. Since the same *Eco*RI, *Bam*HI and *Hin*dIII fragments hybridized with both the Vα2 and Vα7 specific probes, we conclude that one member of the Vα2 subfamily is closely linked to one member of the Vα7 subfamily. **M15S and M15T**. When compared with the M10T lymphoma, both the M15S and M15T lymphomas have retained one additional member of the Vα1, Vα4, Vα5, Vα8, Vα10 and Vα11 subfamilies and two additional members of the Vα2, Vα3 and Vα7 subfamilies. Moreover, relative to the M15S cell line, the M15T cell line displays an extra Vα11 germline fragment. Both M15S and M15T have rearranged a Vα4 and a Vα11 gene segment. However, the rearranged Vα4 and Vα11 gene segments are different in each case (see Figure 2). Southern blot of *Hin*dIII-digested DNA shows that the M15T cell line displays a rearranged Vα9 hybridizing band with a size identical to the one containing the rearranged Vα4 gene (see Figure 2) and the correspondingly rearranged Jα gene segment (data not shown). This last result suggests that the rearranged *Hin*dIII fragment contains at least one member of the Vα9 and Vα4 subfamilies. Finally, the M5T lymphoma has rearranged the Vα5T gene segment and displayed a second rearrangement detectable with both the Vα2 and Vα7 probes. Again, the fact that a single rearranged *Bam*HI, *Eco*RI or *Hin*dIII fragment hybridized with both the Vα2 and Vα7 probes indicates that some members of these two subfamilies are closely linked. In addition, Southern blot analysis reveals that the M5T T lymphoma has only lost one member of the VαBM.B subfamily as well as the entire Vα6 and Vδ1 subfamilies and retained germline REF patterns in 16 out of the 18 Vα subfamilies tested.

Table II. Germline restriction fragments detected with probes specific for fifteen distinct Vα subfamilies in 13 T lymphoma clones and subclones.

Probes[a]	Germline[b] restriction fragments		T Lymphoma											
	Enzyme	Size	M5T	M15T	M15S	M10T	M8T	M8T7	M14T	M14T1	M14T7	M14T2	M14T8	M31T	M31T2
Vα1	BamHI	12.9	+	+	+										
		10.0	+	+	+	+	+	+	+	+	+	+	+	+	+
		8.0	+	+	+	+	+	+	+	+	+	+	+	+	+
		7.8	+	+	+	+	+	+	+	+	+	+	+	+	+
		6.0	+	+	+	+	+	+	+	+	+	+	+	+	+
		4.5	+	+	+	+									
		4.4	+	+	+	+									
		3.0	+	+	+	+	+								
		2.8	+	+	+	+	+	+	+						
		2.4	+	+	+	+	+	+	+	+	+	+	+	+	+
		1.7	+	+	+	+	+	+	+	+	+	+	+	+	+
Vα2	EcoRI	12.4	+												
		8.5	+	+	+	+	+	+							
		6.5	+	+	+										
		5.6	+	+	+	+	+	+	+						
		4.8	+	+	+	+	+								
		3.4	+	+	+										
		3.2	+												
Vα3	EcoRI	16.0	+	+	+										
		11.1	+	+	+	+	+	+	+						
		10.5	+	+	+	+	+	+	+	+	+	+		+	
		8.0	+	+	+	+	+	+							
		5.9	+	+	+	+	+	+							
		4.6	+	+	+	+	+	+	X	X	X	X	X	X	X
		2.1	+	+	+	+	+	+							
Vα4	EcoRI	11.8	+	+	+	+	+	+	+						
		8.7	+	+	+	+									
		3.2	+	+	+	+	+								
		1.8	+	+	+	+	+	+	+	+	+	+			
Vα5	BamHI	12.8	+	+	+										
		8.2	+	+	+										
Vα7	BamHI	25.0	+	+	+										
		19.0	+	+	+	+	+	+							
		15.0	+	+	+	+	+	+	+						
		4.5	+	+	+										
		2.8	+												
		1.7	+	+	+	+	+								
Vα8	HindIII	19.6	+	+	+	+	+	+							
		16.4	+	+	+										
		4.8	+	+	+	+									
		4.4	+	+	+	+	+	+							
		3.4	+												
		2.3	+	+	+	+	+	+	+						
Vα9	HindIII	16.5	+												
		7.8	+	+	+	+	+	+							
Vα10	BamHI	15.5	+	+	+										
		10.9	+	+	+	+	+	+							
		8.3	+												
		6.6	+	+	+	+	+	+	+						
		6.2	+												
		5.1	+	+	+	+	+	+							
		2.7	+	+	+										
Vα11	HindIII	23.0	+	+	+										
		16.4	+	+	+	+	+	+	+						
		5.8	+	+	+	+	+	+							
		3.8	+												
Vα13	EcoRI	4.8	+												
		4.2	+	+	+	+	+	+	+	+	+	+	+	+	+
		3.1	+	+	+	+	+	+	+	+	+	+	+	+	+
Vα14	EcoRI	2.0	+	+	+	+	+	+	+	+	+				
Vα1BMA	BamHI	9.4	+	+	+	+	+	+	+	+	+	+	+	+	+
		7.4	+	+	+	+									
		3.5	+	+	+	+	+	+							
		3.2	+	+											
Vα1BMB	EcoRI	5.9	+	+	+	+	+								
		4.2	+												
Vα1BWB	EcoRI	12.6													
		6.6	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]The Vα probes are described in Table I.
[b]The lengths of restriction fragments detected by Southern blot hybridization were determined with standard fragments run in parallel and are indicated in kb.
+Indicates the retention in a given T lymphoma of a restriction fragment in germline configuration.
X indicates the polymorphic restriction fragments of CB20 strain detected in the M14T clone and its subclones with the Vα3 probe.

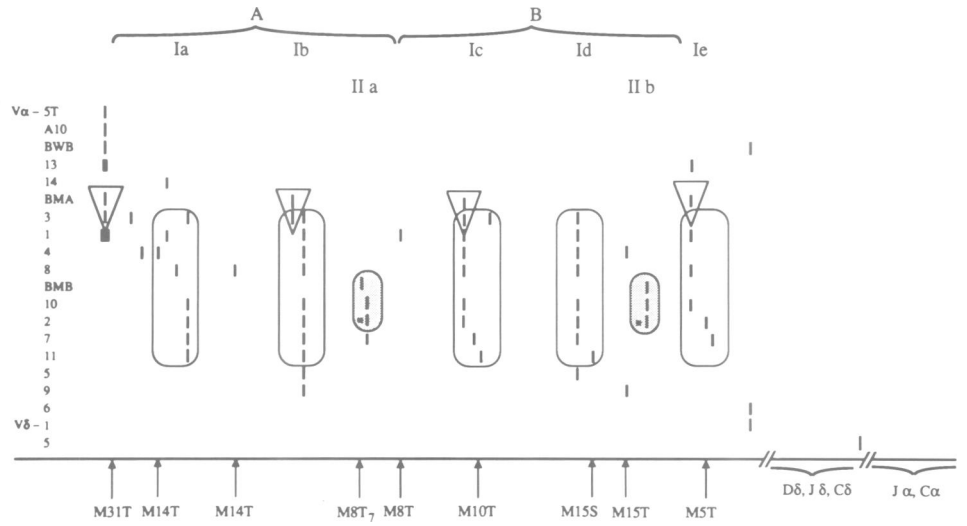


Fig. 2. Relative order of the members of 18 Vα and two Vδ gene segment subfamilies. The localization of the different V gene segments present in the $V\alpha^a$ haplotype is indicated relative to the Dδ−Jδ−Cδ−Jα−Cα gene elements. Actual physical distances are unknown. The names of the Vα gene segment probes that have been used in Southern blot analyses are indicated on the left. Each solid rectangle represents a Vα- or Vδ-related gene segment and corresponds to a discrete hybridizing band identified using Southern blot analyses. One cluster composed of two Vα13 segments and one cluster containing six Vα1 segments have been depicted by increasing the length of the corresponding solid rectangles. Vertical arrows below the horizontal line indicate the positions of some of the informative rearrangement breakpoints used to establish the deletion map. The relative order of the V gene segments mapped between the T cell line deletion breakpoints cannot be determined from these analyses. Consequently, the corresponding segments have been aligned on a vertical line. Repetitive patterns in the V gene segment distribution are indicated by differently shaped boxes. Analysis of the chromosomal distribution of the Vα gene segments allows the definition of three distinct Vα clusters (see Results). Cluster I is repeated at least four times (Ia−Id) and contains one member of each of the Vα1, Vα2, Vα3, Vα4,. Vα7, Vα8, Vα10 and Vα11 subfamilies. Cluster II is repeated twice (IIa and IIb) and contains one member of each of the Vα2, Vα10 and VαBM.B subfamilies. The Vα2 gene segments present in this second cluster are marked with an asterisk to indicate that they specifically hybridize to probe W (see Results). Finally, cluster III is repeated at least three times (inverted triangles) and contains one member of each of the Vα3 and VαBM.A subfamilies. The $V\alpha^a$ region may be divided into two repeated subregions denoted as A and B (see Results). Also shown is the Vδ5 gene segment which has been localized in the opposite transcriptional orientation, 2.5 kb 3′ to Cδ (Iwashima *et al.*, 1988; Korman *et al.*, 1988).

two distinct repeats of the Vα8-Vα10-Vα2-Vα7 motif (class 1 and 3) and one Vα1-Vα8-Vα10-Vα2-Vα7 unit (class 1). The latter is totally identical, both in terms of the nature and relative order of the segments, to a large section of the Vα cluster I. Three out of the nine identified Vα2 classes have been found to hybridize to probe W (classes 5, 6 and 9 in Figure 3) and display the Vα10-ProbeW-Vα2 motif. Moreover, the VαBM.B-Vα10-ProbeW-Vα2 unit present in the fifth class was found to be identical to the set of Vα gene segment postulated to define the Vα cluster II. Most of the V gene segments identified within a cluster, appear to be separated from their next neighbour by < 10 kb of intervening DNA. Futhermore, in cases where the transcriptional orientation has been determined, all the members belonging to a given cluster have been found to lie in the same polarity.

Therefore, our results show unequivocally the existence within the $V\alpha^a$ and $V\alpha^b$ haplotypes of at least two distinct and variably repeated Vα clusters which contain Vα gene segments belonging to different subfamilies. Such an organization makes it very likely that blocks of DNA carrying more than one V gene segments (i.e. the above defined Vα clusters) have been duplicated several times over the course of α-chain gene evolution. The following evolutionary pathway may be tentatively proposed to explain the present-day organization. Divergence of the two ancestral Vα clusters I and II was followed by a first round of duplication involving the cluster I only. The resulting unit (I-I′-II) has then undergone a second duplication event leading to the Ia-Ib-IIa-Ic-Id-IIb motif depicted in Figure 2 and to the possibility of individualizing two Vα subregions denoted as A (Ia-Ib-IIa) and B (Ic-Id-IIb). This ancestral organization may have been blurred by transposition, recombination or less extended amplification events. For instance, a set of at least five contiguous Vα1 gene segments is located at the distal end of the locus, and lies outside one of the cluster I repeats. Interestingly, the Vα and Vδ subfamilies containing a single or a few members (Vα5T, VαA10, VαBW.B, Vα13, Vα6, Vδ1 and Vδ5) are located at one or both extremities of the V locus and may consequently have been spared from the previously postulated duplication events. Finally, it should be noted that the large degree of Vα gene segment interspersion of the mouse Vα gene segment family outlined in this study may also contribute to the understanding of TCR α gene polymorphism. For instance, we have recently observed that the $V\alpha^e$ haplotype found in the RIII/Rd mouse strain probably results from a single unequal recombination event between the $V\alpha^a$ and $V\alpha^b$ haplotypes. In that process, a block of 11 different $V\alpha^b$-derived segments has been gained by the $V\alpha^a$ haplotype (Jouvin-Marche *et al.*, 1989). Interstrain polymorphism may also involve variation in the numbers of cluster repeats. For instance, three Vα10-ProbeW-Vα2 unit repeats are present in the $V\alpha^b$ haplotype (Figure 3), whereas only two repeats of the corresponding Vα cluster II are found in the $V\alpha^a$ haplotype (Figure 2). Similar examples of V gene segment and cluster duplications are found in the immunoglobulin heavy (Blankenstein and Krawimkel, 1987; Rathbun *et al.*, 1987; Berman *et al.*, 1988; Brodeur *et al.*, 1988) and light (Pech *et al.*, 1984, 1985) chain loci, as well as in the TCR β chain locus (Chou *et al.*, 1986).

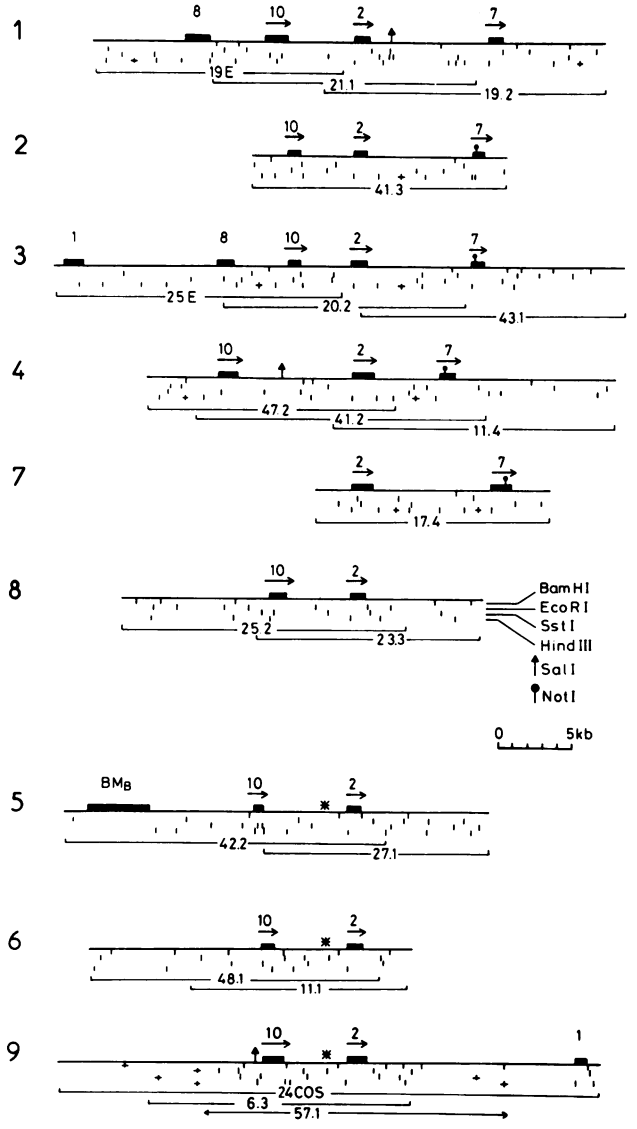


Fig. 3. Restriction maps of the Vα2 subfamily and of the neighbouring Vα1, Vα7, Vα8, Vα10 and VαBM.B gene segments. Phages were isolated from a B10.A liver DNA library using a Vα2-specific probe and mapped for the indicated enzymes by single and double digestions. Based on overlapping restriction maps, representative phage clones were organized in nine distinct classes. The nine composite maps were aligned relative to a common *Hin*dIII restriction site contiguous with the first exon of each Vα2 gene segment. *Bam*HI, *Eco*RI, *Sst*I and *Hin*dIII cleavage sites are indicated in that order below the horizontal lines. A + between two restriction sites indicates the presence of additional recognition sites in this region for the same enzyme. The *Not*I cleavage sites present in the Vα7-containing phages and the *Sal*I cleavage sites are indicated above the horizontal lines. The identified Vα gene segment coding regions are indicated by solid boxes above the horizontal lines. The arrows above the coding regions indicate 5′ to 3′ orientation. In cases where the 5′ to 3′ direction has not been determined, no arrow is given. The asterisks above the horizontal lines indicate the presence of Vα2 5′ flanking sequences hybridizing to probe W (see Results). Also shown is a cosmid clone (24COS) isolated using a Vα2 probe from a B10.WR7 liver DNA library (Malissen *et al.*, 1988). This clone was shown to overlap with the ninth phage class and extended the corresponding map to the 3′ side of the identified Vα2 gene segment. The integrity of the nine composite maps has been verified by hybridizing Southern blots of B10. A liver DNA with Vα2-, Vα7- and Vα10-specific probes (Figure 4 and data not shown). The relative sizes of the unique Vα2 hybridizing *Eco*RI fragment present in each Vα2 class have been used as an arbitrary numbering basis (see Figure 4).

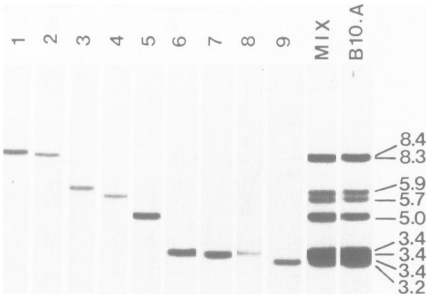


Fig. 4. Reconstruction of genomic Southern blot from representative phages containing gene segments of the Vα2 subfamily. Representative phages corresponding to each of the nine Vα2 classes depicted in Figure 3 were separately digested with *Eco*RI. 0.5 ng (one equivalent genome) of each of the nine *Eco*RI phage digests were either mixed (lane Mix) or separately (lanes 1–9) electrophoresed in parallel with 10 μg of *Eco*RI-digested B10.A liver DNA (lane B10.A) and after transfer to nitrocellulose, hybridized to a Vα2-specific probe (see Materials and methods). The relative sizes (in kb) of the Vα2 hybridizing *Eco*RI fragments are indicated in the right margin.

Some human immunoglobulin Vκ gene segments have been found outside the κ locus (Lötscher *et al.*, 1986), and one Vβ and one Vδ gene segment are located downstream of their corresponding C genes (Malissen *et al.*, 1986; Iwashima *et al.*, 1988; Korman *et al.*, 1989). These observations have led us to wonder if the majority of the Vα gene segments identified in this study maps inside the TCR α/δ genetic complex and upstream of the Cα gene. In the absence of a complete physical map, the best evidence in favour of the fact that most of the identified Vα/δ gene segments are linked on the same chromosome and located upstream of the Cα gene stems from the observation that the M31T-2 cell line has retained in germline configuration only 10 out of the 72 identified Vα hybridizing fragments (see below).

Based on usage frequency, the mouse Vα family has been estimated to comprise approximately 75–100 gene segments (review in Davis and Bjorkman, 1988). In the present study, we have identified a total of 72 hybridizing fragments on Southern blots of BALB/c liver DNA digested with *Eco*RI, *Bam*HI or *Hin*dIII and separately hybridized with a panel of 18 subfamily-specific probes. Three lines of argument indicate that the latter number should be considered as a minimal estimation of the germline content. First, coincidental comigration of Vα-containing restriction fragments may lead to an underestimation of the actual content of Vα gene segments. For instance, nine distinct Vα2 genomic copies have been isolated from a B10.A liver library (Figure 3), whereas only six to seven distinct Vα2 containing REFs have been observed on Southern blots of BALB/c and B10.A liver DNAs digested with *Eco*RI, *Bam*HI or *Hin*dIII (see Figure 4 and Urban *et al.*, 1988). Along the same line, the presence of some additional members of the Vα3, Vα4 and Vα11 subfamilies located in the 3′ most proximal region of the Vα/δ locus and possibly contributing to a fifth Vα cluster I (Ie in Figure 2) may have been similarly masked. Secondly, after longer exposure or less stringent washing conditions, some of the Vα probes used in this study and specific for the Vα4, Vα5, Vα9 and Vα11 subfamilies were shown to detect additional hybridizing bands. However, short of cloning and sequencing the corresponding bands, it is presently impossible to ascertain unambiguously whether

they correspond to additional divergent members of the Vα3, Vα4, Vα5 or Vα11 subfamilies or result from a low level of cross-hybridization to members of other Vα subfamilies. Thirdly, the existence of additional, as yet uncharacterized Vα gene segment subfamilies is still possible. Regardless of these limitations, the determination of the size of the functional germline Vα/δ repertoire will still require the number of V pseudogene segments within each subfamily to be taken into consideration. A limited number of studies have already indicated the presence of pseudogene segments in the Vα3, Vα5, Vα8, Vα9 and VαBW.B subfamilies (Arden *et al.*, 1985; Chou *et al.*, 1986; Letourneur and Malissen, 1989).

Our data also bear on the relationships existing between the Vα and Vδ gene segments. Analysis of the nucleotide sequences of the V gene segments involved in the constitution of δ chain genes indicates that some of them display >75% homology to known Vα gene segments and accordingly may be classified as *bona fide* members of the Vα2 (Okazaki and Sakano, 1988; Happ *et al.*, 1989; Takeshita *et al.*, 1989), Vα4 (Korman *et al.*, 1988), Vα6 (Elliott *et al.*, 1988), Vα7 (Elliott *et al.*, 1988; Okazaki and Sakano, 1988; McConnell *et al.*, 1989; Takeshita *et al.*, 1989), Vα10 (Takagaki *et al.*, 1989; Takeshita *et al.*, 1989), Vα11 (Bluestone *et al.*, 1988) and VαBM.B (Takeshita *et al.*, 1989; D.Couez and B.Malissen, unpublished results) subfamilies. Conversely, other δ-associated V segments (e.g. Vδ1, Vδ2, Vδ4 and Vδ5; Elliott *et al.*, 1988) differ from Vα sequences about as much as most Vα subfamilies differ from each other. Studies of the stages of intrathymic T cell development have revealed that the δ chain genes begin rearranging first on day 14 of gestation and precede by 2–3 days the onset of α chain gene rearrangements. In the mouse, analyses of the fetal and neonatal immunoglobulin repertoires have revealed that V_H gene segment usage displays a position-related bias, with J_H-proximal V_H gene segments used at a high frequency (review in Alt *et al.*, 1987). Similarly, the TCR Vδ gene segments may correspond to a subset of Vα gene segments that are proximal to the Dδ–Jδ–Cδ region and thus likely to rearrange early in ontogeny (Elliott *et al.*, 1988). The results of our deletion mapping clearly indicate that the single-membered Vδ1 and Vα6 (Vδ3) subfamilies are closer to the Dδ–Jδ–Cδ region than any of the 16 other subfamilies tested (Figure 2). Likewise, the Vα1, Vα2, Vα7, Vα8 and Vα10 related segments found in δ chain genes (Happ and Palmer, 1989) may have been exclusively located within the 3′ most proximal cluster I repeat (denoted as Ie in Figure 2). However, analysis of the extrachromosomal circular DNA molecules found in BALB/c thymocytes has revealed that at least two distinct Vα2 and Vα7 gene segments are capable of being implicated in δ gene rearrangements (Okazaki and Sakano, 1988). When considered together with the fact that a single Vα2–Vα7 pair is present in repeat Ie (Figure 2), these results suggest that the δ gene rearrangements involving Vα2- or Vα7-related gene segments may not exclusively feed on the 3′ most proximal Ie repeat. Similarly, the presence of a VαBM.B related gene segment in at least one Vδ gene (Takeshita *et al.*, 1989; D.Couez and B.Malissen, unpublished results), indicates that δ gene rearrangements may also involve members of the cluster II (Figure 2). Therefore, the fact that a given V gene segment will be preferentially targeted to the Dδ–Jδ or Jα gene segments may not only depend on its relative location. For instance, the recent identification of negative *cis*-acting DNA elements (silencers) controlling the differential expression of the TCR α (Winoto and Baltimore, 1989) or TCRγ loci (Bonneville *et al.*, 1989) in the γδ and αβ lineages suggests that V gene segment-associated silencers may also contribute to control their differential expression and rearrangement in the two lineages. Obviously, linking the Vα/δ gene segments to the Dδ–Jδ–Cδ region, positioning the δ *rec* sequences (Takeshita *et al.*, 1989) relatively to the Vα/δ segments and determining the regulatory sequences controlling the expression of each of the positioned Vα/δ gene segments will be needed to understand fully the mechanisms accounting for the programmed pattern of Vα/δ gene segment usage and shed light on the lineage relationships existing between the αβ and γδ T lymphocyte subsets.

Materials and methods

Cell lines

The obtention and the characterization of the seven T lymphomas and corresponding subclones have been previously reported (Primi *et al.*, 1988; Marolleau *et al.*, 1988). Briefly, the lymphoma lines were obtained by *in vivo* retroviral transformation of thymocytes of either BALB/c or C.B20 mouse strain. The C.B20 strain is a congenic BALB/c strain with a chromosome 12 of C57BL/6 origin. After being adapted to culture, the cell lines were cloned by limiting dilution and for each line one clone was randomly selected for further investigations. During *in vitro* propagation of the selected M8T, M14T and M31T cloned cell lines, subclones were derived by limiting dilution on thymocyte feeder cells (Marolleau *et al.*, 1988). When checked by Southern blot hybridization with DH- and Cβ-specific probes, each of the subclones was shown to display the same IgH and TCR β patterns of rearrangement as the parental line (Marolleau *et al.*, 1988).

DNA probes

The characteristics of the 18 Vα and of the single Vδ probes used in this study are indicated in Table I. Analysis of the patterns of hybridization obtained on BALB/c liver (germline) DNA digested with either *Bam*HI, *Eco*RI or *Hin*dIII indicates that the 18 Vα probes detect non-overlapping sets of restriction enzyme fragments (REF) and therefore allows the unambiguous identification of the members of 18 different non-cross-hybridizing Vα subfamilies (Figure 1 and Table II). As shown in Table II, the number of members of each of these Vα subfamilies varies from one to 12. In some cases, depending on the enyzme used, we have observed variations in the number of REF detectable with a given Vα probe. For example, in the BALB/c genome three fragments were detected with the VαBM.B probe by using *Bam*HI digests, whereas only two bands were visible by using *Eco*RI digests (see Figure 1). The M5T cell line is the only one to have retained a complete set of germline VαBM.B REF. Along the same line, two hybridizing bands were observed with the Vα5 probe using *Bam*HI digests, whereas only one strong hybridizing band was seen using *Eco*RI digests. The two Vα5 hybridizing bands detectable on *Bam*HI digests were retained only in the M15S, M15T and M5T lymphomas. Probe W corresponds to a 900 bp *Kpn*I fragment isolated from clone 58.1 KB5 (Hue *et al.*, 1990). The orientations of some of the Vα2, Vα7 and Vα10 gene segments shown in Figure 3 were determined using (i) a 600 bp *Bam*HI–*Eco*RV fragment isolated from clone 42.2 (see Figure 3) and corresponding to Vα2 5′ flanking and coding sequences, (ii) a 200 bp *Eco*RV–*Bam*HI fragment isolated from clone 42.2 and corresponding to Vα2 3′ coding sequences, (iii) an 800 bp *Eco*RI–*Hin*dIII fragment isolated from clone 41.2 (see Figure 3) and corresponding to Vα7 5′ flanking sequences, (iv) a 170 bp *Eco*RI–*Not*I fragment isolated from a Vα7 cDNA clone (E.Palmer, unpublished results) and corresponding to Vα7 5′ coding sequences, (v) an 80 bp *Not*I–*Eco*RI fragment isolated from the same Vα7 cDNA clone as above and corresponding to Vα7 3′ coding sequences, (vi) a 600 bp *Pvu*II–*Sst*I fragment isolated from clone 28.1 J6.19 (Hue *et al.*, 1990) and corresponding to Vα10 5′ flanking sequences and (vii) a 200 bp *Sst*I–*Pvu*II fragment isolated from clone 28.1 J6.19 and corresponding to Vα10 coding sequences. Probes were labelled by random priming (Feinberg and Vogelstein, 1983) to 1.2×10^8 c.p.m./g DNA.

Southern blot analyses

High mol. wt DNAs were isolated according to Maniatis *et al.* (1982) with minor modifications. Briefly, cells (5×10^7) were resuspended in 2 ml 50 mM Tris pH 7.5, 10 mM EDTA, 0.2 M NaCl, lysed by addition of 0.1 ml 5% SDS and incubated for 3–16 h with 0.2 mg/ml proteinase K. Proteins were removed by extraction with an equal volume of phenol. DNA was precipitated with 2.5 vol of ethanol. Restriction enzyme digestion, gel electrophoresis and blotting procedures were performed as previously described (Morgado *et al.*, 1989). Filters were hybridized overnight in a hybridization mix solution containing 3 × SSC, 10× Denhardt's solution, 0.1% SDS, 1 mM EDTA, 10% dextran sulphate and 1 mg/ml of herring sperm DNA plus the labelled probe. After hybridization, the filters were first washed with 3 × SSC (Standard Sodium Citrate), 0.05% SDS for 15 min at room temperature, then in 1 × SSC, 0.5% SDS for 15 min at 65°C and finally with 0.1 × SSC, 0.05% SDS for 30 min at 65°C. The filters were autoradiographed at −80°C in the presence of intensifying screens. Filters were used several times, after removing the probe via 30 min incubation at 45°C in 0.4 M NaOH followed by neutralization in 0.1 × SSC, 0.1% SDS, 0.2 M Tris–HCl pH 7.5, at 45°C.

To locate the Vα7 related gene segments present in the phages corresponding to the nine Vα2 classes (Figure 3), filters were washed with 1 × SSC, 0.05% SDS for 30 min at 50°C.

In the Southern blot reconstruction experiment shown in Figure 4, the amounts of DNA loaded in each lane have been homogenized by adding 10 μg of sonicated salmon sperm DNA to the *Eco*RI phage digests electrophoresed in lanes 1–9 and Mix.

Construction and screening of genomic phage library

The B10.A genomic library was constructed from *Sau*3A partially digested liver DNA using the bacteriophage vector EMBL3 (Frischauf *et al.*, 1983) according to Maniatis *et al.* (1982). Screening was performed as previously described (Malissen *et al.*, 1986).

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